Kidney International, Vol. 41 (1992), pp. 542–545

SIGNAL TRANSDUCTION

Cytosolic and nuclear signaling by endothelin peptides: Mesangial response to glomerular injury

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Cell signaling plays an important role in directing the phenotypic changes that mesangial cells undergo in glomerular disease. These changes include increased synthesis and reorganization of extracellular matrix, protease secretion, processing of extracellular macromolecules such as immune complexes, and cell growth in the form of hypertrophy and hyperplasia [1]. Although rearrangements or mutations in the mesangial genome might account for some of these phenotypic alterations, it is now clear that many of these changes result from regulation of gene expression by extracellular ligands. Proinflammatory ligands, such as growth factors, cytokines, and peptides, evoke a network of short-term signals that are extended to long-term events involving differential regulation of gene expression. But despite recent advances, the pathways of signaling by which these ligands regulate the genetic program of mesangial cells remain unclear.

To understand how extracellular stimuli regulate mesangial phenotype, we have focused on the recently described peptide hormones, endothelins (ET) [2]. ET are a widely expressed family of 21-amino acid peptides with diverse biological actions [reviewed in 2, 3]. ET peptides are expressed at numerous sites in the kidney, but glomerular ET is thought to arise mostly from the glomerular endothelium [4] and from mesangial cells themselves [5, 6]. Secreted ET binds to receptors on the mesangial cell plasma membrane and initiates a cascade of signals that cause both short-term and long-term actions [7]. Short-term actions are directed primarily at the regulation of mesangial cell contraction [8–11] and secretion [8]. But the fact that ET is a potent mitogen in mesangial cells [12] reveals that ET also stimulates differential gene expression. ET has already been shown to increase expression of genes for the c-*fos* protooncogene [12] and for the platelet-derived growth factor A and B chains [13]. Thus, cultured mesangial cells provide a valuable model to study the pathway of signaling from cytosol to nucleus and the possible implications for glomerular disease.

The pathways of ET signaling are becoming clear, although obvious gaps in our understanding remain. Figure 1 summarizes a working model of the signal transduction pathways activated by ET in mesangial cells. We first briefly describe the cytosolic second messengers generated by interaction of ET with plasma membrane receptors. Finally, we will explain how these cytosolic signals regulate the AP-1 family of inducible trans-activators, thereby controlling the genetic program of mesangial cells.

Receptors for ET and short-term signals

The pathway of signal transduction in mesangial cells is initiated by binding of ET ligands to integral membrane receptors of two different subtypes [14–16]. These receptors can be classified experimentally on the basis of differential affinity for ET ligands [14, 15] and by cross linking and electrophoretic separation [16]. ET receptors belong to the G protein-coupled receptor family and share many features with other well-characterized members of this family such as the rhodopsin, adrenergic, and muscarinic receptors [17, 18]. ET receptors, like other G protein-coupled receptors, couple to numerous effector systems via the action of G proteins [19]. To date, ET receptors have not been cloned in mesangial cells, and cloning of receptor subtypes will enable studies aimed at structure/function relationships, regulation of expression, the role of ET receptors in desensitization, and perhaps the rational design of specific receptor blockers.

At least two mechanisms account for the diversity of cytosolic signals emanating from ET receptors (Fig. 1) (see [3] for a thorough discussion of ET signaling). Different receptor subtypes appear to activate distinct signaling systems. In addition, single G protein-coupled receptors interact with multiple G proteins, which can in turn regulate different effectors. In mesangial cells the lower-affinity ET receptor couples directly to many signaling systems including phospholipases C, D and A_2, and directly or indirectly to Ca^{2+} channels and Na^+/H^+ antiporters [8–12]. These signals mediate both the contraction of mesangial cells and secretion of bioactive prostaglandins [8]. In contrast, the high-affinity receptor appears to open a ligand-gated Ca^{2+} channel, but the mechanism and biological significance of this response remains unclear. However, this high-affinity receptor subtype might mediate a hypertrophic response in mesangial cells (Simonson and Dunn, unpublished observations).

Nuclear signaling by ET peptides

We next turn to the question of how the short-term signals discussed above are extended to regulate gene expression in the nucleus. To regulate the expression of target genes, ET must somehow alter the activity of transcriptional proteins involved

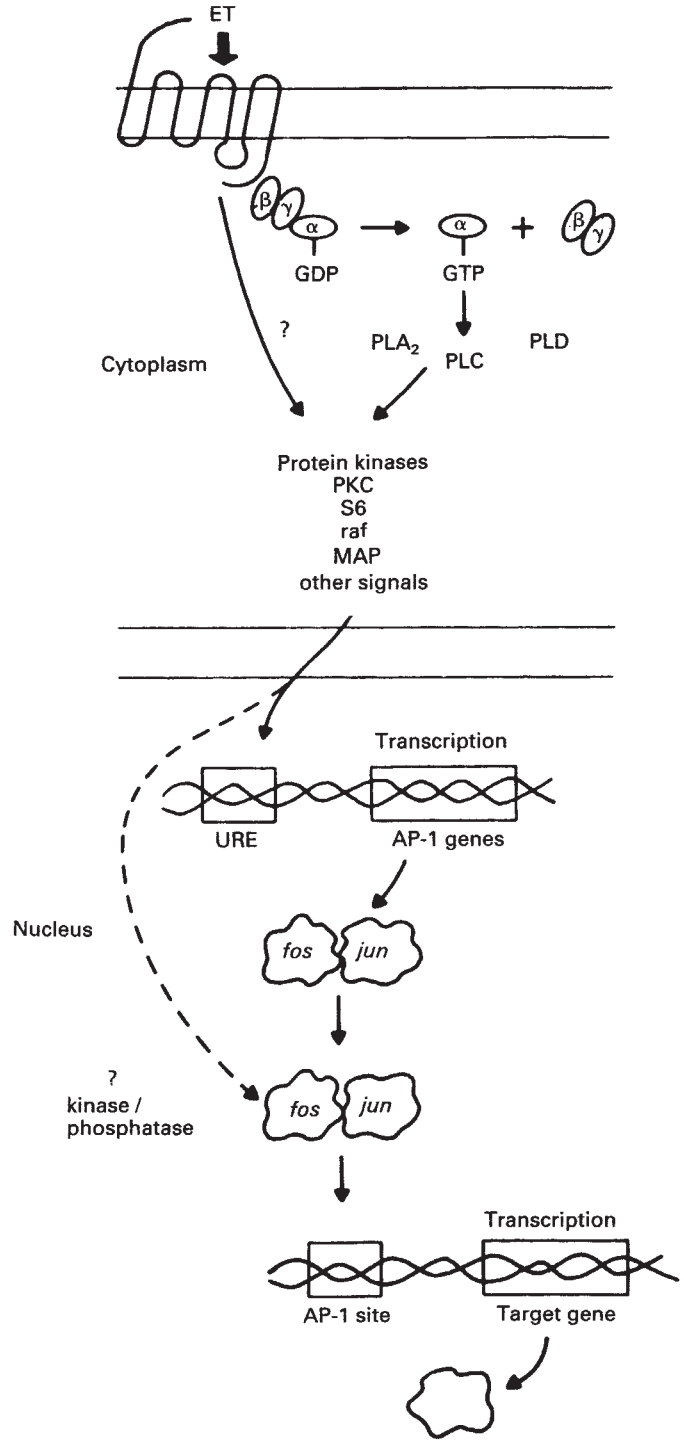

Fig. 1. *Pathway of signal transduction from membrane to nucleus following activation of ET receptors.* ET ligands bind to G protein-coupled receptors in the plasma membrane. Activated ET receptors interact with heterotrimeric G proteins and promote both the exchange of GTP for GDP and the dissociation of GTP-bound alpha subunit. A complicated network of effectors is then activated by the free alpha subunit, resulting in production of multiple second messengers including inositol (1,4,5)P_3, diacylglycerol, prostaglandins, phosphatidic acid, etc. These second messengers may activate as yet unidentified protein kinases that extend these cytosolic signals to the nucleus. These signals ultimately result in activation of upstream regulatory elements (URE) in AP-1 genes to stimulate transcription and the production of various AP-1 dimers. The dotted line indicates that AP-1 complexes can also be regulated post-translationally. The net effect of AP-1 induction is to direct the transcription of target genes (that is, delayed genes) and alter the genetic program of mesangial cells.

in the RNA polymerase II complex, coactivators, or trans-acting factors. Current evidence suggests that gene expression in response to environmental signals occurs by regulating the activity of inducible trans-acting factors. AP-1 (activator protein-1) complexes are prototypes for factors that couple receptor-generated second messengers to long-term responses requiring differential gene regulation [reviewed in 20–22]. Induction of AP-1 factors contributes to diverse phenotypic responses including cell growth and differentiation. In short, AP-1 complexes function as a switch to coordinate the genetic program of cells with changing extracellular signals.

AP-1 consists of a mixture of homodimers and heterodimers that bind to *cis*-acting elements (TGACTCA or close variants thereof) in upstream promotor or enhancer regions of genes. Interactions of bound AP-1 with unidentified components of the basal transcriptional apparatus regulate the initiation rate of RNA polymerase II. AP-1 complexes are encoded by two families of closely related genes: (i) the *fos* family including c-*fos*, *fos* B, and *fra*-1 [23]; and (ii) the *jun* family including c-*jun*, *jun* B, and *jun* D [21]. *Fos* and *jun* monomers then dimerize to form functional homo- or heterodimer AP-1 complexes, and dimerization is required for sequence-specific DNA binding. Dimerization of *fos* and *jun* proteins occurs through a leucine zipper motif that consists of a periodic repetition of leucine residues at every seventh position [24]. Thus, the composition of AP-1 isolated from nuclear extracts consists of variable dimer complexes [22, 25, 26]. These dimers not only have different affinities for the AP-1 binding site but are also thought to have different trans-activating potential.

There is abundant evidence that the magnitude and pattern of AP-1 gene expression regulates AP-1 activity. Induction of AP-1 genes not only controls the concentration of these factors but also regulates in part the relative composition of AP-1 dimers. The composition of AP-1 dimers has important consequences for AP-1 action. For example, different dimers have variable affinities for AP-1 *cis*-acting elements, with *fos*:*jun* heterodimers having greater affinity that *jun*:*jun* homodimers [21, 27]. Moreover, qualitative and quantitative differences in DNase-1-hypersensitivity sites between different AP-1 dimers suggest that these proteins differ in size and shape [22]. It is also possible that distinct AP-1 dimers have different affinities for groups of closely related AP-1 sites in the promoters of target genes. In addition, distinct AP-1 dimers might bind to similar elements in separate genes but have different trans-activational effects on the basal transcriptional machinery [22, 28]. For example, c-*fos*:c-*jun* dimers activate transcription whereas c-*fos*:*jun* B complexes appear, under some conditions, to repress transcription [28]. Thus, regulation of gene expression for AP-1 complexes is an important determinant of AP-1 activity.

To investigate whether AP-1 might mediate some of the

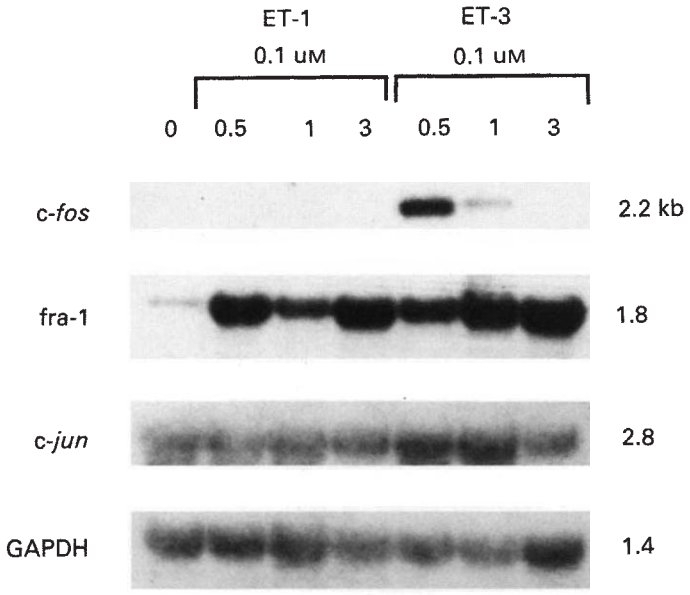

Fig. 2. *ET-1 and ET-3 evoke differential induction of mRNA abundance for AP-1 genes.* Quiescent rat mesangial cells were treated at zero time with 0.1 uM ET-3 or ET-1. At the times indicated, total RNA was extracted, fractionated in agarose gels, and transferred to nitrocellulose. The steady-state mRNA levels were then analyzed by Northern RNA blot analysis using [^{32}P]-labeled cDNA probes for specific AP-1 genes. The same blot was rehybridized with a cDNA probe for glyceraldehyde phosphate dehydrogenase (GAPDH) to control for differences in RNA loading and for global changes in RNA polymerase II activity.

nuclear events stimulated by ET, we asked if ET differentially regulates AP-1 gene induction in mesangial cells. ET isopeptides, ET-1 and ET-3, markedly stimulate the steady-state mRNA levels for several AP-1 genes including c-*fos*, *fra*-1, and c-*jun* (**Note added in proof;** Fig. 2). Several points regarding the AP-1 response are noteworthy. Induction of AP-1 expression occurs rapidly after the addition of ET and is consistent with a role in cell signaling. The expression of some AP-1 genes such as c-*jun* and *fra*-1 are sustained while genes such as c-*fos* are only transiently induced. The rapid reversal of c-*fos* mRNA abundance is likely due to negative autoregulation of c-*fos* transcription by AP-1 complexes [29], which is thought to limit the accumulation of *fos* in response to incoming signals. In addition, ET-1 and ET-3 differentially regulate AP-1 induction, which might help explain the different biological effects of these peptides (**Note added in proof**). ET-1 stimulates all three AP-1 genes, whereas ET-3 fails to stimulate c-*fos* induction and stimulates c-*jun* and *fra*-1 to a lesser extent than ET-1. Taken together, these experiments suggest that AP-1 complexes contribute to the control of gene expression by ET peptides. Experiments are now underway to investigate the mechanisms by which ET regulates trans-activation by AP-1.

Do other ET-stimulated pathways regulate AP-1 activity? AP-1 activity is controlled at the transcriptional, post-transcriptional, and post-translational levels [21], and it is possible that ET acts at all levels. AP-1 are phosphoproteins, and reversible phosphorylation/dephosphorylation plays an important role in regulating AP-1 activity. For example, in response to protein kinase C c-*jun* is activated by site-specific dephosphorylation catalyzed by a protein phosphatase [30]. By contrast, Ha-Ras amplifies trans-activation by c-*jun* via phosphorylation of its activation domain [31]. AP-1 proteins also bear multiple O-linked N-acetylglucosamine monosaccharide residues [32], although the functional significance of glycosylation is not clear. At present it is unknown whether ET regulates AP-1 through post-translational modifications.

Regulation of the mesangial cell genetic program by AP-1

What are the biological consequences of AP-1 induction by ET? AP-1 genes belong to the immediate-early response genes, which regulate the network of genes responsible for long-term changes such as proteases, receptors, ion channels, cytoskeletal proteins, and other trans-acting factors. At present, little is known about the biological consequences of AP-1 induction in mesangial cells. The best evidence in support of AP-1 action in mesangial cells is induction of the collagenase gene by ET (**Note added in proof**). The collagenase promoter contains an AP-1 *cis*-acting element and is a well-characterized target for transcriptional regulation by AP-1. Because protease secretion contributes to the development of glomerular inflammation, it is tempting to speculate that in glomerulonephritis AP-1 elements mediate increased protease secretion. In addition, AP-1 factors have been implicated in various aspects of cell growth including mitogenesis, differentiation, and development [25]. Our preliminary studies suggest that induction of *fos* proteins correlates with a hypertrophic response in mesangial cells whereas induction of *jun* proteins correlates with a hyperplastic response. Clearly, the identification of late response genes in mesangial cells that are direct targets for AP-1 factors is of pressing importance. These experiments should be facilitated by the identification of AP-1 elements in the promoters or enhancers of target genes and by a detailed analysis of AP-1 activity in mesangial cells.

Concluding remarks

Despite much progress, many questions regarding the pathway of signal transduction from membrane receptor to the nucleus remain: 1) What is the nature of ET receptor genes in mesangial cells and how is their expression regulated? 2) What intermediary protein kinases (that is, Raf, MAP, S6, etc.) contribute to the transcriptional and post-translational regulation of AP-1 in response to ET? 3) What are the target genes responsive to AP-1 factors? 4) What other inducible trans-acting factors play a role in nuclear signaling by ET? 5) How is the AP-1 signaling pathway turned off, and can we develop pharmacological tools to interdict these pathways? Future efforts aimed at analyzing the regulation of AP-1 and other inducible trans-acting factors is likely to provide significant insight into the mechanisms controlling mesangial phenotype in glomerular inflammation.

Note added in proof

SIMONSON MS, JONES JM, DUNN MJ: Differential regulation of *fos* and *jun* gene expression and AP-1 *cis*-element activity by endothelin isopeptides: Possible implications for mitogenic signaling by endothelin. *J Biol Chem* (in press)

Acknowledgments

This work was supported by National Institutes of Health Grant HL-22563 and DK-41684.

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